LOCALIZATION OF ONCOSTATIN-M EXPRESSION IN APICAL PERIODONTITIS LESIONS USING IMMUNOHISTOCHEMICAL ANALYSIS

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CERTIFICATE

This is to certify that this dissertation titled **"LOCALIZATION OF ONCOSTATIN-M EXPRESSION IN APICAL PERIODONTITIS LESIONS USING IMMUNOHISTOCHEMICAL ANALYSIS"** is a bonafide record of work done by **Dr. MANIVANNAN.M** under our guidance during his postgraduate study period between 2009-2012.

This dissertation is submitted to **THE TAMIL NADU Dr.M.G.R.MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY – CONSERVATIVE DENTISTRY AND ENDODONTICS, BRANCH IV**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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INTRODUCTION

The term 'apical periodontitis' is generally used to describe and group together, the various periapical conditions that originate from necrosed or diseased pulp. Any break in the integrity of the external tooth surface by dental caries, chemical erosion, or cracks etc. may result in irritation of the pulp.[39] In these situations the bacterial elements may reach the pulp and cause a tissue response. Without proper treatment, the pulp's inflammatory processes may spread and eventually pulp necrosis will occur. In conjunction with this spread of inflammation through the apical foramina, apical periodontitis may be induced.

According to Fabricius et al, the infected and necrotic root canal system offers a selective environment for causative organisms, which grow mostly in sessile biofilms and also as aggregates, co-aggregates and planktonic cells suspended in the fluid phase of the canal. Collectively this polymicrobial community residing in the root canal system has several biologic and pathogenic properties, such as antigenicity, chemotaxis and activation of host cells (Nair et al).

The microbial invaders in the root canal can advance or their by-products can infiltrate the periapex. In response the host mounts a

dynamic defensive system consisting of several classes of cells, intercellular messengers, antibodies and effector molecules. The clash of the microbial and host defense forces destroys much of the periapical tissues. As a consequence of the microbial infection of the root canal system and the inability of the host defense responses to eliminate the antigens, periapical lesions such as apical abscesses, granulomas and cysts may be formed with the aim of restricting microbes.[23]

Due to the encasement of the root in bone and the relatively greater resistance of the root to resorption, the production of an inflammatory infiltrate usually occurs at the expense of the surrounding bone. The changes in mineralization and structure of the bone adjacent to the site of inflammation form the basis of radiographic diagnostic procedures for the detection and monitoring of apical periodontitis.[14]

Although the defensive reaction minimizes the spread of the infection, it cannot eliminate microbes that are well entrenched in the sanctuary of the root canal system as protected biofilms. Therefore apical periodontitis is not self healing. Resolution requires surgical or non surgical endodontic therapy.

The universal goal of the root canal treatment is to prevent or cure apical periodontitis, caused by the infection of the root canal

system. When the treatment is done properly, healing of the periapical lesion usually occurs with hard tissue regeneration, that is characterized by reduction of the radiolucency on follow-up radiographs.

Periradicular surgery, when indicated, should be considered as an extension of nonsurgical treatment, because the underlying etiology of the disease process and the objectives of treatment are the same: prevention and elimination of apical periodontitis.[40] Even when surgical treatment is the likely definitive approach, nonsurgical therapy before the procedure may be recommended to help reduce the number of microorganism in the root canal system and ensure a more favorable long term prognosis.[40]

Periapical radiolucencies persist when treatment procedures have not reached a satisfactory standard for the control and elimination of the infection. Problems that lead to persistent apical periodontitis include: inadequate aseptic control, poor access cavity design, missed canals, inadequate instrumentation, debridement and leaking temporary or permanent restorations. Even when the most stringent procedures are followed, apical periodontitis may still persist as asymptomatic radiolucencies, because of the complexity of the root canal system formed by the main and accessory canals, their ramifications and

anastomoses where residual infection can persist.[22] In addition, there are factors located beyond the root canal system within the inflamed periapical tissue, that can interfere with the post-treatment healing of the lesion [Nair & Schroeder 1984].

Due to the persistent microbial infection and the increased inflammatory response, periapical lesions tend to grow within the jaw bone. It is understandable that intense bone resorption produced by activated osteoclasts may favor the intraosseous expansion of the lesion.[26] Very little is currently known about the precise mechanisms of the growth of apical periodontitis lesions.

Oncostatin M(OSM) is a pleiotropic cytokine that is produced by activated T cells and macrophages at the time of inflammation.[16]The glycoprotein is structurally and functionally related to IL-6, leukemia inhibitory factor (LIF), and IL-11, proteins that also influence immune and inflammatory function. OSM regulates cell growth and differentiation in a wide variety of biological systems, including hematopoiesis, neurogenesis and osteogenesis.[6] Numerous activities have been ascribed to OSM expression in vitro, including the differentiation of megakaryocytes, inhibition of tumor cell growth, induction of neurotrophic peptides, regulation of cholesterol

metabolism, and effects on bone-derived cells.[37]There is a strong evidence implicating that OSM can participate in bone remodeling. Moonga et al studied the effects of OSM in isolated rat osteoclasts.[20] Richards et al (2000) have demonstrated the role of OSM in the induction of osteoclast differentiation and resorptive activity in the mouse. OSM is regarded as the proinflammatory cytokine that may be responsible for the bone resorption associated with apical periodontitis.

The aim of the present study was to investigate the expression of OSM in persistent apical periodontitis lesions by immunohistochemical analysis.

The objective of the present study was:

1. To investigate the expression of OSM in persistent apical periodontitis lesions
2. To compare the OSM expression in apical periodontitis lesions of size greater than 5 mm and size less than 5 mm.

REVIEW OF LITERATURE

Kakaheshi et al (1965)[15] reviewed the influence of viable micro-organisms on the fate of a surgically exposed dental pulp. The study was undertaken using germ free animals. He showed that the integrity of an unexposed dental pulp can be maintained after severe injury only in the absence of a bacterial infection. He concluded that the presence or absence of microbial flora is the major determinant in the healing of exposed rodent pulps.

Mikkonen M et al (1983) investigated healing after periapical surgery by studying clinical and radiolographic features in 108 patients in whom apicoectomy was performed. Histologic diagnosis was used to determine healing results. He found that teeth with periapical granuloma tend to heal less successfully than teeth showing inflammation or cysts. Postoperative treatment played an important role in the healing process. He explained age of the patient also has some influence on healing.

Lustmann et al (1991) studied the relation of pre-operative and intraoperative factors to prognosis of posterior apical surgery. The relation of preoperative and intraoperative factors to the prognosis of apical surgery was studied in 136 premolar and molar roots. The evaluated factors were: clinical signs and symptoms, size of the

periapical lesion, root canal obturation, tooth restoration, apical retrofilling, and the operating surgeon. He found a significantly higher success rate in roots obturated 2 mm or shorter of the apex, in roots without post restoration, and after retrofilling of roots with apparently well-condensed obturation.

Bellido et al (1995)[2] reviewed the functional receptor complexes assembled in response to interleukin-6 and -11 (IL-6 and IL-11), leukemia inhibitory factor (LIF), oncostatin M (OSM), and ciliary neurotrophic factor (CNTF). IL-6 and IL-11 induce homodimerization of gp130, while the rest heterodimerize gp130 with other gp130-related beta subunits. Some of these cytokines (IL-6, IL-11, and CNTF) also require a specificity-determining alpha subunit not directly involved in signaling. He concluded that stromal/osteoblastic cells are targets for the actions of all the members of the cytokine subfamily that shares the gp130 signal transducer and suggested that different receptor repertoires may be expressed at different stages of differentiation of this lineage.

Bruce et al (1996)[6] reviewed Oncostatin M (OSM) cytokine whose structural and functional features are similar to other members of the interleukin (IL)-6 family of cytokines, many of which utilize gp130 as a common receptor subunit. A biologically active OSM receptor has

been previously described that consists of a heterodimer of leukemia inhibitory factor receptor (LIFR) and gp130. This LIFR gp130 complex is also a functional receptor for LIF. He cloned and characterized an alternative subunit (OSMRb) for an OSM receptor complex (a heterodimer of gp130 and OSMRb) that is activated by OSM but not by LIF. He demonstrated that both LIF and OSM cause tyrosine phosphorylation and activation of the gp130 LIFR combination, but the gp130-OSMRb complex is activated by OSM only. He concluded that OSM-induced cellular responses, initiated through low affinity binding to gp130, are mediated by two heterodimeric receptor complexes that utilize alternative signal transducing subunits that confer different cytokine specificities to the receptor complex.

Danin J et al (1996) compared the outcome of retreatment and surgical intervention in root canal treated teeth with nonhealing periradicular pathosis. He found that the success rate for surgery was higher than for conventional retreatment, but the difference was not statistically significant. He concluded that for management of nonhealing periradicular pathosis associated with root canal treated teeth, surgical intervention should be considered as an alternative to retreatment.

Wang hui et al (1997)[12] measured OSM in synovial fluid found in patients with rheumatoid arthritis and osteo-arthritis. He found that OSM was detected in 18 or 20 samples of synovial fluid from rheumatoid arthritis patients. There was no detectable OSM in synovial fluid from osteo-arthritis patients. He concluded that OSM may contribute to joint inflammation in rheumatoid arthritis.

Bohic et al (1998)[3] reviewed the effects of LIF and OSM on the physicochemical characteristics of mineral phase formed in a rat bone-marrow stromal cell culture model. He analysed the physicochemical characteristics of the mineralization nodules formed were analyzed by energy dispersive X ray microanalysis (EDX) and Fourier transform infrared (FT-IR) and FT-Raman spectroscopy. EDX and FT-IR spectroscopy revealed the influence of LIF and OSM on the physicochemical characteristics of mineral phases. FT-Raman spectroscopy showed modifications of the main vibrational modes of the organic matrix. He concluded that these alterations induced by growth factors could help define new strategies for the prevention and treatment of skeletal disorders.

Alan grenier et al (1999)[9] investigated the potential production and regulation of OSM by human polymorphonuclear neutrophils. He

found that OSM secretion occurs through a two step mechanism in PMN, consisting of early release of a preformed stock, followed by denovo protein synthesis. This would allow rapid and sustained OSM release to occur at an inflammatory site and may contribute to the modulation of local inflammation.

Gomez et al (1999)[7] reviewed the action of oncostatin over wide variety of cells and its diversified biological responses in vivo and in vitro. He suggested the potential role of oncostatin in the regulation of complex cellular processes such as gene activation, cell survival, proliferation and differentiation. OSM is a multifunctional cytokine that is structurally and functionally related to the sub family of cytokines IL-6. OSM shares properties with all members of this family but is most closely related to LIF. He concluded that OSM and LIF can bind to the same functional receptor complex and thus mediate overlapping spectra of biological activities.

Lisignoli et al (1999)[18] demonstrated both in vitro and ex vivo the role of mature osteoblasts (OB) and bone marrow stromal cells (BMSC) in Rheumatoid arthritis(RA) and Osteo-arthritis(OA) by analysing the expression of the following IL-6-type cytokines: IL-11, leukaemia inhibitory factor (LIF), oncostatin M (OSM) and IL-6.The

evolution of OA and RA is characterized by processes of bone remodelling: in OA, subchondral bone stiffness increases, while erosions and osteonecrosis are typical features of RA bone. OB and BMSC were isolated from femora of RA, OA and post-traumatic (PT) patients, cultured in vitro in the presence or absence of IL-1b and tumor necrosis factor-alpha (TNF-a), and assessed for the production and mRNA expression of IL-6-type cytokines. He demonstrated that IL-6-type cytokines are constitutively expressed in the bone compartment in RA, OA and PT patients and can be secreted by bone cells at different stages of differentiation (BMSC and OB). This suggests that these cytokines may be involved in the mechanisms of bone remodeling in OA and RA.

Marc vase et al (1999)[35] analyzed the effect of oncostatin M (OSM) on angiogenesis, as it could be involved in the development of atherosclerosis. The effect of OSM was compared with those of leukemia inhibitory factor (LIF) and interleukin-6(IL-6). On human dermal microvasculature endothelial cells (HMEC-1s), OSM (22.5 to 112.5 pmol/L) induced a dose-dependent increase in cell proliferation greater than that induced by the classic angiogenic factors vascular endothelial growth factor and basic fibroblast growth factor (Bfgf), LIF induced only a 30% increase in cell proliferation, and IL-6 had no effect.

He concluded that OSM induces an angiogenic effect on capillary endothelial cells, which could be, at least in part, implicated in pathological processes such as atherosclerosis or tumor growth.

Samuel Varghese et al (1999)[34] reviewed the effects of Leukemia inhibitory factor (LIF) and oncostatin M (OSM) on skeletal remodeling. He tested whether LIF and OSM regulate the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in osteoblasts enriched cells isolated from fetal rat calvariae. He concluded that LIF and OSM stimulate collagenase-3 and TIMP-1 expression in osteoblasts and these effects may be involved in bone remodeling actions of these cytokines.

Wallace et al (1999)[37] reviewed OSM, which is a pleiotropic cytokine produced late in the activation cycle of T lymphocytes and macrophages. He showed that administration of OSM inhibited bacterial LPS-induced production of TNF and lethality in a dose dependent manner. Consistent with these findings, OSM potently suppressed inflammation and tissue destruction in murine models of rheumatoid arthritis and multiple sclerosis. He concluded that the activities of this cytokine in vivo are anti-inflammatory without concordant immunosuppression.

Bruce et al (2000)[6] reviewed OSM which is a pleiotropic cytokine regulating cell growth and differentiation in a wide variety of biological systems, including hematopoiesis, neurogenesis and osteogenesis. The elaboration of the biological activities of OSM has been confounded by the presence of different OSM receptor signaling systems in humans. He concluded that OSM signals threw two different receptor complexes-the LIF/OSM shared receptor, which shares high affinity binding with LIF, a protein with structural similarity to OSM and the OSM specific receptor which binds OSM uniquely

Carrie Langdon et al (2000)[16] reviewed OSM that can regulate a number of connective-tissue cell types in vitro including cartilage and synovial tissue-derived fibroblasts, however its role in joint inflammation in vivo is not clear. He analyzed murine OSM (mu OSM) activity in vitro and in vivo in mouse joint tissue, to determine the potential role of this cytokine in local joint inflammation and pathology. He concluded that OSM regulates synovial fibroblast function differently than other IL-6-type cytokines, and can induce a proliferative invasive phenotype of synovium in vivo in mice on overexpression. He suggested that OSM may contribute to pathology in arthritis.

. **Heymann et al (2000)[11]** reviewed the role of gp130 cytokine family in bones cell differentiation or activation and in osteo-articular pathologies. This family included IL-6, IL-11, OSM, LIF, cilliary neurotropic factors and cardiotropin. He reported the role of IL-6 in bone destruction in malignant hypercalcemia. IL-11 may induce the formation of osteolytic bone metastasis. LIF and OSM promoted cartilage degradation in rheumatoid arthritis patients. He concluded that detection of gp130 family cytokines in osteo-articular pathologies is a definitive indication of the importance in these disease processes.

Richards et al (2000)[25] demonstrated the role of OSM in the induction of osteoclast differentiation and resorptive activity in the mouse. He examined the ability of recombinant OSM to stimulate osteoclast formation and compared the responses to other members of the IL-6 family of cytokines including mouse leukemia inhibitory factor, cardiotropin-1 and IL-6. He concluded that mouse OSM, cardiotropin-1 and leukemia inhibitory factor induce osteoclast differentiation and activation.

Wahl et al (2001)[36] suggested that OSM is anabolic, anti inflammatory, and promotes wound healing. In models of inflammation OSM is produced late in the cytokine response promoting the re-

establishment of homeostasis by co-operating with pro inflammatory cytokines and acute phase molecules to alter and attenuate the inflammatory response. In animal models of chronic inflammatory disease OSM potently suppressed inflammation and tissue destruction. T cell function and antibody production were not impaired by OSM treatment. He indicated that activities of this cytokine in vivo or anti-inflammatory without concordant immunosuppression.

Moonga et al (2002)[20] compared the effects of IL-6, OSM, leukemia inhibitory factor on bone resorption and cytosolic calcium signaling. He concluded that IL-6R activation by IL-6 stimulates osteoclastic bone resorption either by reversing the inhibitory effect of high extracellular calcium in stromal containing systems or itself stimulating bone resorption along with calcium ions by micro-isolated osteoclasts

Palmqvist et al (2002)[24] studied the bone resorptive effects of IL-6, OSM and leukemia inhibitory factor and their regulation of receptor activator of NF-Kb ligand(RANKL),RANK, osteoprotegrin (OPG) in neonatal mouse calvaria .He demonstrated stimulation of calvarial bone resorption and regulation of mRNA and protein expression of RANKL & OPG by IL-6 family cytokines.

Sisko Huumonen et al (2002)[14] reviewed the advantages and limitations of the radiological examination in periapical diagnosis. He suggested that diagnosis and management of periapical pathosis requires a thorough clinical and radiographic examination. Along with normal radiographic techniques other imaging techniques like tomography, computed tomography, Magnetic resonance imaging, Ultrasound and nuclear techniques have been reviewed. He concluded that radiographic diagnosis of chronic apical periodontitis is a complex task, which is confounded by several anatomical and biological variables. Between the extremes of well-defined, normal periapical structures and pathognomonic radiolucencies, detection and grading of radiographic signs of chronic apical periodontitis may be difficult. Systems for training and calibration of observers may be used to improve diagnostic performance, and digital manipulations have great potential for the detection of subtle changes indicating disease.

Spence et al (2002)[29] revealed OSM – mediated expression of m-RNA's encoding tissue type plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1). Northern blot analysis showed that the level of tPA – mRNA expression is nearly undetectable in calu-1 cells. Exposure of these cells to OSM for 20 hours increased tPA – mRNA expression by twenty fold and PAI-1 mRNA expression

by five fold. He showed that plasminogen activating system has shown to participate in degradation of bone like matrix and implicated OSM has a mediator of bone destruction.

Takafumi et al (2002)[30] measured the protein levels of OSM in the supernatants of dendritic cells cultures by ELISA and examined the expression of OSM mRNA by RT-PCR after stimulation with lipopolysaccharide (LPS) or fixed *Staphylococcus aureus*. He concluded that human monocyte-derived DCs can synthesize and secrete large amounts of OSM in response to bacterial products, suggesting that OSM produced by DCs at infectious sites may play a role in modulating inflammatory responses.

Tsai et al (2002)[32] reviewed the role of COX 2 which is a key enzyme in prostaglandin synthesis, in the pathogenesis of pulpal or periapical lesions. He investigated the in situ localization of COX 2 in radicular cysts using immunohistochemistry. A peroxidase-labelled strepdavidin-biotin technique was used for the identification of COX 2. He concluded that the COX 2 expression is significantly higher in radicular cysts and COX 2 played an important role in the pathogensis of radicular cysts.

Nair et al (2004)[23] reviewed the pathogenesis of apical periodontitis and the causes of endodontic failures. He viewed apical periodontitis as a dynamic encounter between microbial factors and host defenses at the interface between the infected radicular pulp and periodontal ligament that results in local inflammation, resorption of hard tissues and eventual formation of various histopathologic categories of apical periodontitis, commonly referred to as periapical lesions. He concluded that even when the highest standards and most careful procedures are followed, failures still occur. He explained that this is because there are root canal regions that are inaccessible by the current equipments and that is the reason for persistent infection.

Shih – junglin et al (2004)[17] investigated the presence of OSM and IL-6 in gingival crevicular fluid. He showed that both OSM and IL-6 were detected in most gingival crevicular fluid samples. The total amounts of IL-6 & OSM were significantly positive correlated with severity of diseased sites. He concluded that both OSM & IL-6 may play a role in modulating the inflammatory cascade of chronic periodontitis.

Wang hui et al (2004)[13] demonstrated that Oncostatin M is a pro-inflammatory cytokine previously shown to promote marked cartilage destruction both in vitro and in vivo when in combination with

IL-1 or tumor necrosis factor alpha. He reviewed the in vivo effects of these potent cytokine combinations on bone catabolism are unknown. Using adenoviral gene transfer, he over expressed oncostatin M in combination with either IL-1 or tumor necrosis factor alpha intra-articularly in the knees of C57BL/6 mice. Both of these combinations induced marked bone damage and markedly increased tartrate-resistant acid phosphatase-positive multinucleate cell staining in the synovium and at the front of bone erosions. Furthermore, there was increased expression of RANK and its ligand RANKL in the inflammatory cells, in inflamed synovium and in articular cartilage of knee joints. He concluded that this model of inflammatory arthritis demonstrates that, in vivo, oncostatin M in combination with either IL-1 or tumor necrosis factor alpha represents cytokine combinations that promote bone destruction.

Goren et el (2006)[8] investigated the role of the cytokine oncostatin M (OSM) for wound biology. OSM and its specific OSM receptor subunit β (OSMRβ) were induced upon injury. OSM induction paralleled the early influx of polymorphonuclear neutrophils (PMN) into the wound. OSM protein was localized in PMN in very early wounds, whereas OSMRβ could be detected on macrophages, keratinocytes, and fibroblasts later in repair. To establish a functional connection between

PMN and OSM expression in wounds, we depleted mice from circulating PMN by injecting an anti-PMN monoclonal antibody (Ly-6G). PMN-depleted wounds were characterized by a nearly complete loss of OSM but not OSMRβ mRNA and protein expression within the initial 16–24 hours after injury. He concluded that there is strong evidence that OSM expression during wound inflammation is functionally connected to PMN infiltration.

Nair et al (2006)[22] reviewed on the causes of persistent apical periodontitis. He explained that even when proper procedures are followed, apical periodontitis may still persist as asymptomatic radiolucencies because of the complexity of the root canal system. He listed the following factors are responsible for persistent infection: [1] intra radicular infection, [2] extra radicular infection, [3] foreign body reaction, [4] presence of true cystic lesions.

Brounais et al (2008)[4] reviewed the in vitro effects of OSM which reduces the growth and induces differentiation of osteoblast and osteo sarcoma cells into osteocytic cells. OSM sensitises these cells to apoptosis. He designed an in vivo study model where adenoviral gene transfer of OSM was done in naïve and osteosarcoma – bearing rats. He analysed the bone variables using CT scanner, by histology and by the

levels of various bone markers. The results show that in osteosarcoma rat model OSM reduced the progression of primary bone tumor. He confirmed that systemic OSM over expression alters the osteoblast/ osteo sarcoma activity.

Benedicte brounais et al (2008)[5] reviewed the in vitro studies on primary osteoblastic and osteosarcoma cells (normal and transformed osteoblasts) and have shown that oncostatin M (OSM), a member of the interleukin-6 family, possesses cytostatic and pro-apoptotic effects in association with complex and poorly understood activities on osteoblast differentiation. In a study he used rat osteosarcoma cells transduced with lentiviral particles encoding OSM to stably produce this cytokine. He concluded that osteosarcoma cells stably producing OSM do not develop resistance to this cytokine and thus could be a valuable new tool to study the anti-cancer effect of OSM in vivo. Moreover, OSM over-expressing osteoblastic cells differentiate into osteocyte-like cells, the major cellular contingent in bone, providing new culture conditions for this cell type which is difficult to obtain in vitro.

Hams et al (2008)[10] reviewed the function of OSM in the face of an inflammatory challenge. He used a peritoneal model of acute inflammation to define the influence of OSM on chemokine-mediated

leukocyte recruitment. When compared with wild-type and IL-6-deficient mice, peritoneal inflammation in oncostatin M receptor deficient (OSMR-KO) mice resulted in enhanced monocytic cell trafficking. In contrast to IL-6-deficient mice, OSMR-KO mice displayed no difference in neutrophil and lymphocyte migration. Subsequent in vitro studies using human peritoneal mesothelial cells and an in vivo appraisal of inflammatory chemokine expression after peritoneal inflammation identified OSM as a prominent regulator of CCL5 expression. He concluded that IL-6 and OSM individually affect the profile of leukocyte trafficking, and they point to a hitherto unidentified interplay between OSM signaling and the inflammatory activation of NF-kB**.**

Tsai et al (2008)[33] investigated the in situ location of OSM in epithelialized apical periodontitis lesions. He demonstrated that OSM stain was detected in the inflammatory infiltrates, epithelium, connective tissue and endothelium. The OSM signal was mainly expressed in endothelial cells (100%) followed by inflammatory infiltrates (93%), epithelial cells (53%) and fibroblasts (16%). He concluded that OSM was found to be expressed in epithelialized apical periodontitis lesions and would form part of the cytokine network involved in the disease process of apical periodontitis.

Zvi Metzger et al (2009)[19] conducted studies on apexum procedure and about its possible role in enhancing healing kinetics of periapical lesions. He performed this study to explore the safety and efficacy of apexum procedure. He found that there was no adverse effects after performing this procedure and the healing kinetics in these lesions were significantly enhanced. He observed a 87% and 95% healing rates after 3 and 6 months respectively.

Manojkumar et al (2010)[31] measured the level of OSM in the gingival crevicular fluid and serum of chronic periodontitis patients. He investigated the correlation between them before and after periodontal therapy. Oncostatin M (OSM) is a gp130 multifunctional cytokine that belongs to the IL-6 subfamily and produced mainly by activated T cells, neutrophils, monocytes, and macrophages. More recently, it has also been demonstrated that OSM is produced by dendritic cells. OSM has been found to fulfill Koch's postulates as an inflammatory mediator. OSM may act alone or synergistically with IL-6/TNF-α stimulating the production of IL-6, to up-regulate the production of MMPs or augment IL-6 production which may cause connective tissue and bone destruction. He found that mean OSM levels were elevated in both GCF and serum of chronic periodontitis patients and these levels were decreased proportionally after the periodontal therapy. He concluded

that increased OSM levels both in GCF and serum, and the decreased levels after initial periodontal therapy may suggest the use of OSM as an inflammatory biomarker in the periodontal disease.

Sims et al (2010)[28] reviewed the function of gp 130 signaling cytokines in inflammation and maintenance of bone homeostasis. Bone remodeling is the process by which bone is continually renewed.This process occurs both within the thick cortical bone that surround the marrow space and on the surface of the internal network of bone(trabecular bone). He concluded that gp 130 cytokines regulate the differentiation and activity of osteoblasts, osteoclasts and chondrocytes.

Carmina Barone et al (2010)[1] conducted the Toronto study to assess the long term outcome of apical surgery performed on root filled teeth presenting with post-treatment apical periodontitis. The author identified three significant outcome predictors- age, preoperative root filling length and size of the surgical crypt. He concluded that the outcome was better in subjects >45 years old, teeth with inadequate root filling length and crypt size ≤ 10mm.

Fan Rong et al (2011)[26] investigated the possible association between the expression of bone resorption regulators (RANKL and OPG) and inflammatory cell infiltration in apical periodontitis. He

observed the number of RANKL positive cells and the ratio of RANKL/OPG in apical periodontitis were significantly higher than those in healthy periapical tissues. He concluded that RANKL expression was increased with T, B Lymphocyte and macrophage infiltration in apical periodontitis, which is a key determinant in periapical bone resorption.

MATERIALS AND METHODS

Armamentarium

- Microtome
- Autoclave
- Hot air oven
- Slide warmer
- Couplin jars
- Measuring jar
- Weighing machine
- APES coated slides
- Slide carrier
- Aluminium foil
- Micro-pipettes
- Toothed forceps
- Electronic timer
- Beakers
- Rectangular steel tray with glass rods
- Sterile gauze

- Cover-slips
- Light microscope
- Periosteal elevators
- Surgical excavators
- XCP film holder
- E-speed X-ray films

Reagents used

- Conc. HCl
- Laxbrosoln
- APES (3 amino propyl tri ethoxvsilane)
- Acetone
- Citrate buffer
- Phospho Buffer Saline (PBS)
- 3% H_20_2
- Deionized distilled water
- Haematoxylin
- Absolute alcohol
- Xylene
- Harri'shematoxylin
- Eosin
- 1% acid alcohol

Antibodies used

1. Primary antibody — oncostatin-m Rabbit polyclonal antibody (SantaCruz)

2. Secondary antibody — Rabbit Avidin Biotin Complex staining system (SantaCruz)

METHODOLOGY

Case selection:

30 patients were selected with radiographic evidence of periapical lesions and with no history of systemic diseases. Initially conventional root canal treatment was attempted in these patients but it had failed. All the patients reported persistent symptomatic pain after initial root canal treatment. All the patients were selected within the age group of 20-40, because this group is less susceptible to systemic diseases.

Exclusion criteria:

Exclusion criteria included the patients with inflammatory bone diseases like rheumatoid arthritis, osteoarthritis and patients with chronic periodontitis. These cases were excluded because expression of

increased oncostatin-M has been detected in inflammatory bone diseases like rheumatoid arthritis and chronic periodontitis.

Radiographic technique:

Parelleling radiographic technique was used to obtain radiographs with minimal image distortion. XCP (Extension Cone Paralleling) film holder with external guiding ring was used. The guiding ring is used to align the x-ray aiming cylinder and ensures accuracy of the radiographic image. Using vernier caliper the mesio-distal diameter of the lesion is measured on the radiograph.

Grouping:

Based on the size of the lesion, the patients were divided into two groups- group I and group II.

GROUP I:

15 patients were selected in this group with lesion size less than 5mm.

GROUP II:

15 patients were selected with lesion size greater than 5mm.

SAMPLE COLLECTION AND STORAGE:

After grouping, periapical surgery was performed in these patients. With the help of surgical excavators, lesions were scrapped out and stored in 10% buffered formalin in a sealed container. The tissue specimen was allowed to fix in formalin overnight and then the following day, the specimen was embedded in paraffin which is made into blocks. One biopsy specimen from inflamed gingiva was used as positive control.

PRETREATMENT OF THE SLIDES

- The slides were first washed in tap water for 2 minutes
- The slides were then soaked in detergent solution for 1 hour
- After 1 hour, each slide was brushed using a scrub with the detergent solution and were transferred to distilled water.
- The slides were washed in two changes of distilled water.
- The slides were then washed in autoclaved distilled water.
- The slides were immersed in 1 N HCL (100 ml HC1 in 900 ml distilled water) overnight.
- The following day slides were taken out of acid and washed in autoclaved distilled water twice.

- All the slides were then transferred to slide trays, wrapped in aluminium foil and baked in hot air oven for 4 hours at 180 degrees centigrade.

APES coating:

The baked slides were then dipped in coupling jar containing acetone for 2 minutes. Following this the slides were dipped in APES (3-Amino Propyl tri Ethoxy Silane) for 5 minutes. The slides were then subjected to distilled water wash for 2 minutes. The slides were left to dry. The slides were coated with APES for better adherence of tissue sections to the slides.

PREPARATION OF PARAFFIN SECTIONS:

After the slides were dry, tissue section of 5 micron thickness were made in a rotary manual microtome. The ribbons of tissue section were transferred onto the APES coated slide from the tissue float bath such that two tissue bits come on to the slide with a gap in between. One of the tissue sections was labeled positive (P) and the other negative (N). For each sample 2 sections were made and transferred to 2 slides. One

slide was processed for immunohistochemistry and the other slide was stained with eosin and hematoxylin.

HEMATOXYLIN AND EOSIN STAINING:

The slides were dewaxed and hydrated through graded alcohol to water. The sections on the slides were flooded with Harri's hematoxylin for 5 minutes. The slides were washed in running tap water for 5 minutes. The slides were differentiated in 1% acid alcohol for 5 minutes. The slides were washed well in running tap water for 5 minutes. The tissue section on the slides were then stained in eosin for 30 seconds. The slides were washed in running tap water for 1 minute.

STAINING PROTOCOL FOR IHC:

The slides with tissue sections were treated with three changes of xylene to remove paraffin wax. They were put in descending grades of alcohol and then rehydrated with water. Slides were then treated with 3% hydrogen peroxide for 30 minutes to quench endogenous peroxidase activity of cells that would otherwise result in non -specific staining. Then the slides were transferred to citrate buffer and autoclaved for antigen retrieval at 15 lbs pressure for 30 minutes. Then the slides were dipped in 3 changes of distilled water for 5 minutes

each. Circles were drawn around the tissues so that the antibodies added later on do not spread and are restricted to the circle. The tissues were incubated in protein blocking serum for one hour in an enclosed hydrated container. Then the slides were wiped carefully without touching the tissue section to remove excess of blocking serum.

The primary antibody, rabbit polyclonal antibody. 1:50 dilution was added to the tissueP on the slide and then to the N, PBS was added. The slides were incubated for one hour. Then the slides were wiped carefully without touching the tissue section to remove excess of antibody and washed with three changes of cold PBS for 5 minutes. Then a drop of biotin conjugated secondary antibody was added on both the sections and the slides were incubated for 30 minutes. Later slides were washed in three changes of cold PBS for 5 minutes in each. The slides were wiped carefully without touching the tissue section to remove excess PBS. Then a drop of avidin biotin enzyme reagent was added on both the sections and the slides were incubated for 30 minutes. The sections were washed in 3 changes of cold PBS for 5 minutes in each. Then the slides were wiped carefully without touching the tissue section to remove excess PBS. Then a drop of freshly prepared DAB (3-Diaminobenzidine Tetra Hydrochloride a substrate chromogen) was added on both sections. Slides were then washed in

distilled water to remove excess DAB and counter stained with hematoxylin. The slides were placed in a tray with tap water for bluing. Then the slides were transferred to 70% alcohol, 100% alcohol and one change of xylene. The tissue sections were mounted with DPX. The slides were then observed under the microscope. Throughout the procedure care was taken not to dry the tissues.

Consent and Information for Endodontic Surgery

Because non-surgical endodontic treatment is not 100% successful, occasionally inflammation and infection will persist in the bony area around the end of your tooth's root. When this happens, the endodontist may have to perform an apicoectomy. In this procedure the endodontist opens the gum tissue near the tooth to see the underlying bone and to remove any inflamed and infected tissue. The very end of the root is also removed and frequently a small filling may be placed to seal the end of the root canal. Sutures or stitches are placed in the gum to help the tissue heal properly. In about 90% of cases in a period of six to eighteen months the bone will heal around the end of the root. If **periapical surgery** is indicated for the treatment of your tooth, there are possible complications associated with such surgery. These include treatment failure, swelling, discoloration, sensitivity, postoperative infection, jaw muscle cramps and spasm, damage or devitalization of adjacent teeth or roots, and paresthesia

I fully understand the above statements in this consent form. All my questions have been answered. I hereby give my consent to the performance of periapical surgery on the tooth (or teeth) listed above. I further give my consent for the administration of medications,

anesthetics, drugs, and services deemed necessary to treat my endodontic problem, understanding that risks are involved. I consent to color photography of my teeth and to use of the excised tissue during the surgical procedure for scientific thesis or educational purposes in scientific publications or at dental meetings.

_____

Signature of patient / Date

METHODOLOGY

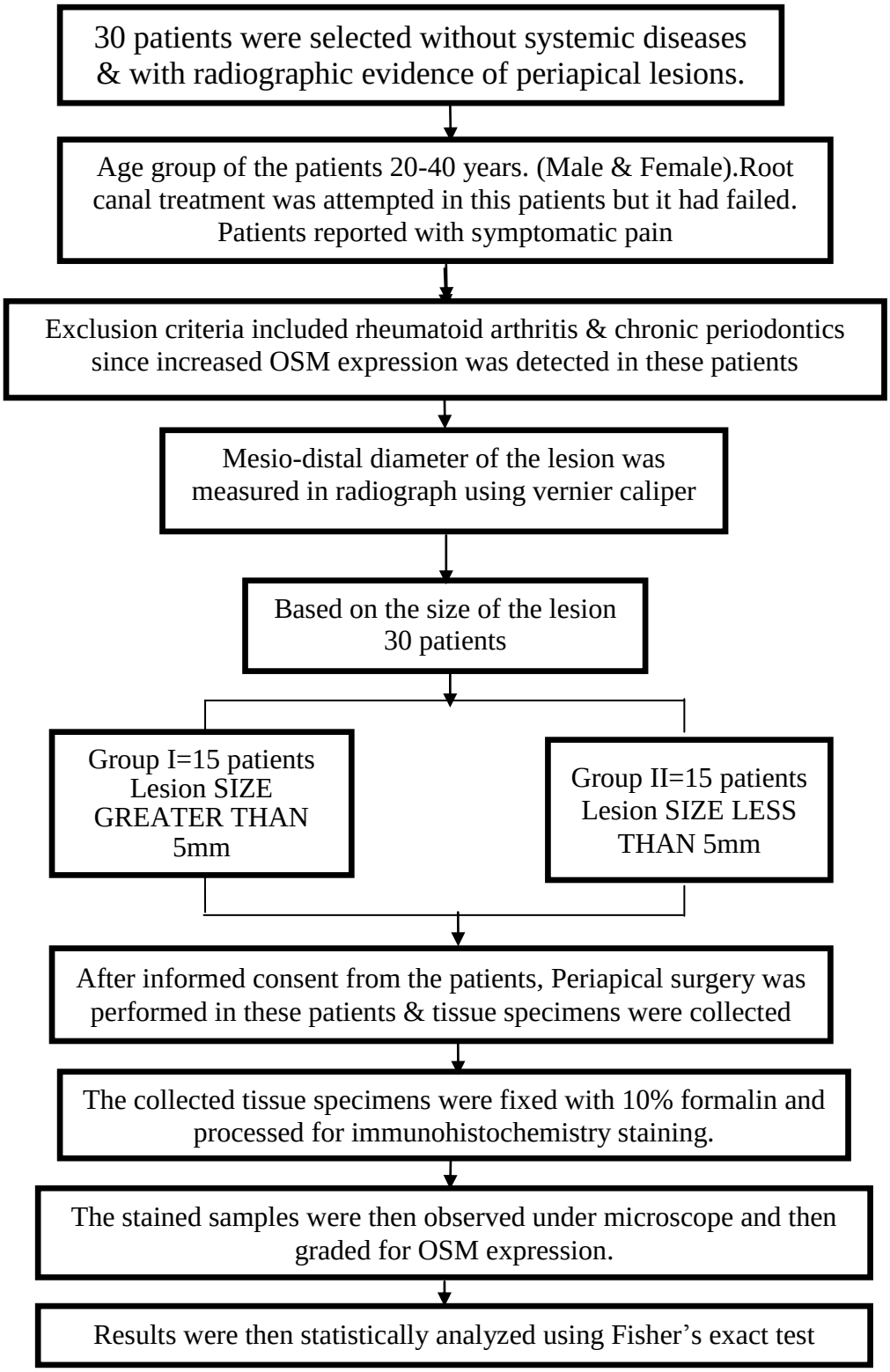


Fig.1: ARMAMENTARIUM

Fig.2: X-Ray Machine

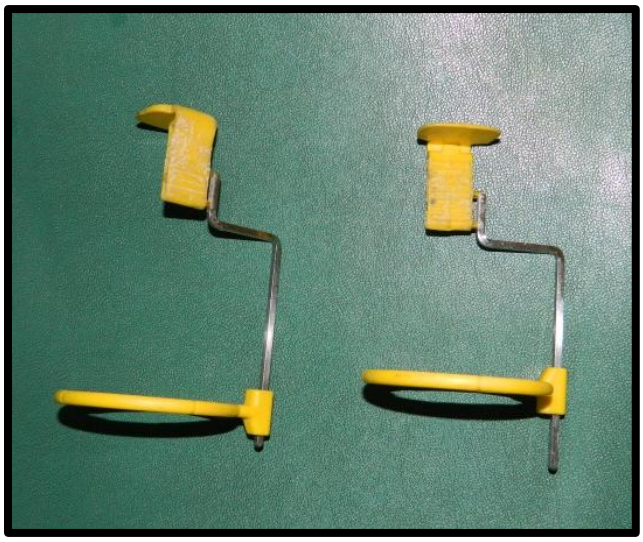


Fig.3: XCP Film holder

Fig.4: E-Speed dental X-ray film

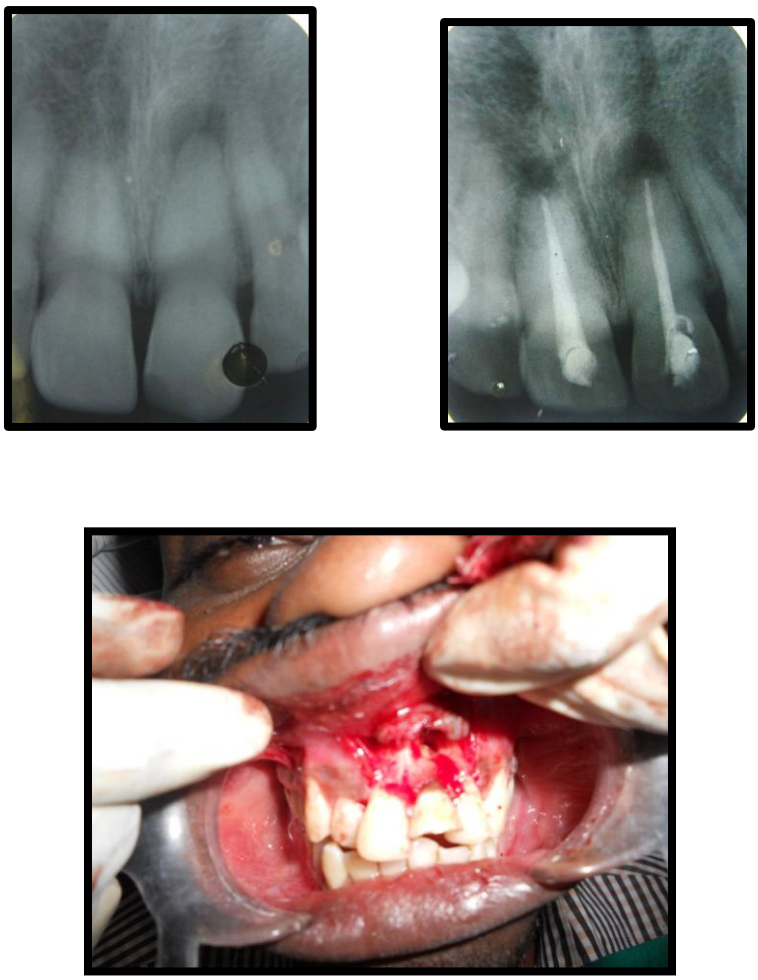


Fig.5: Periapical surgery and tissue specimen collection

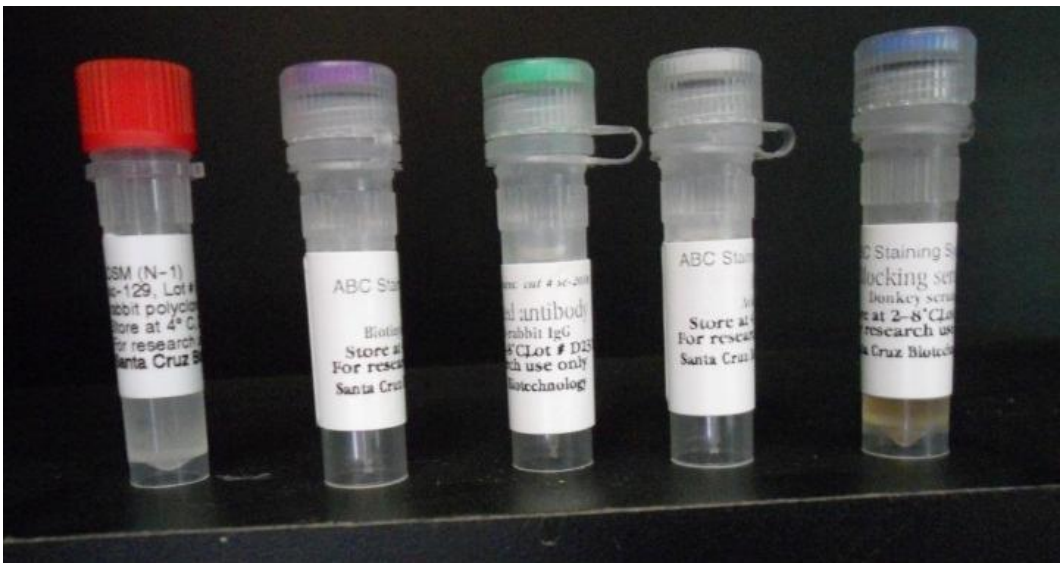


Fig.6: Primary and secondary antibody

Fig.7: Paraffin blocks

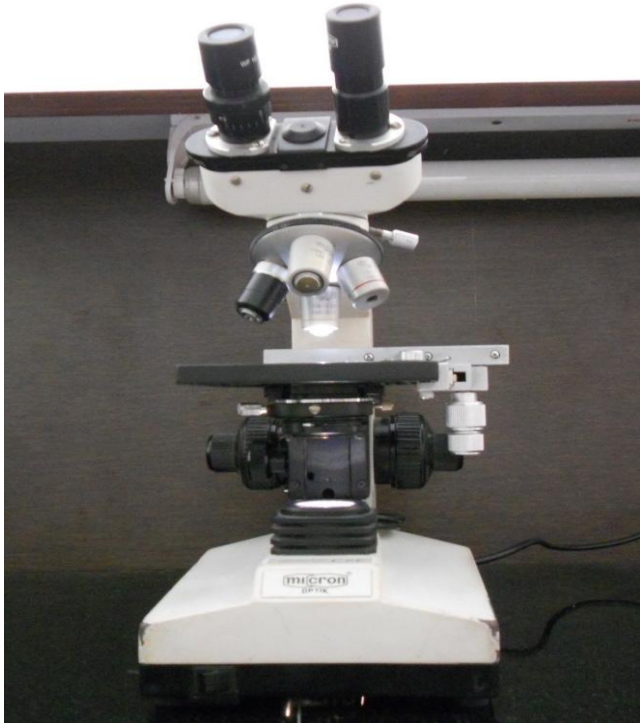


Fig.8: Light microscope

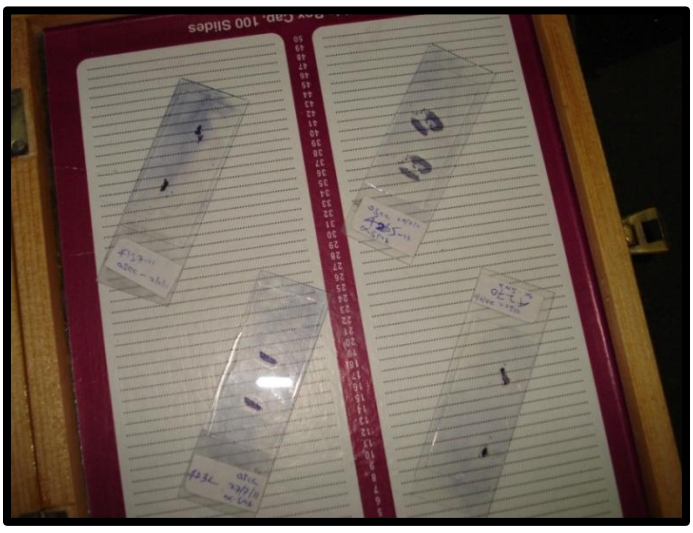


Fig.9: OSM Stained slides

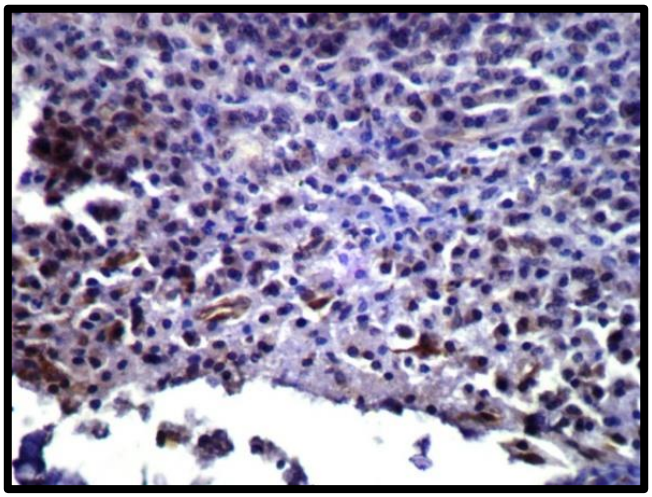


Fig.10: Lesions of size <5mm

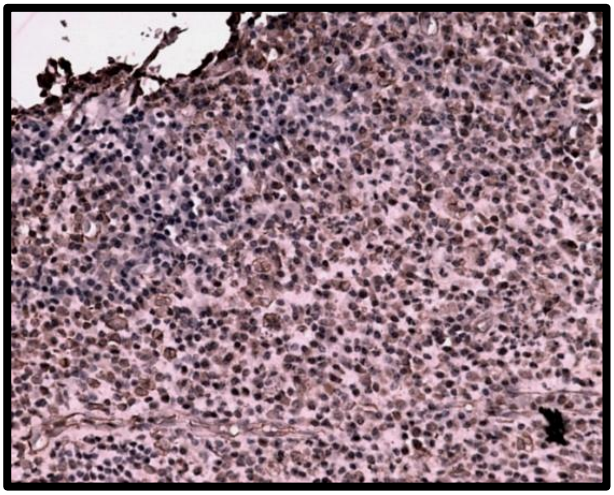


Fig.11: lesions of size > 5mm

High OSM expression was observed in lesion of size >5mm. OSM positive cells were stained brown.

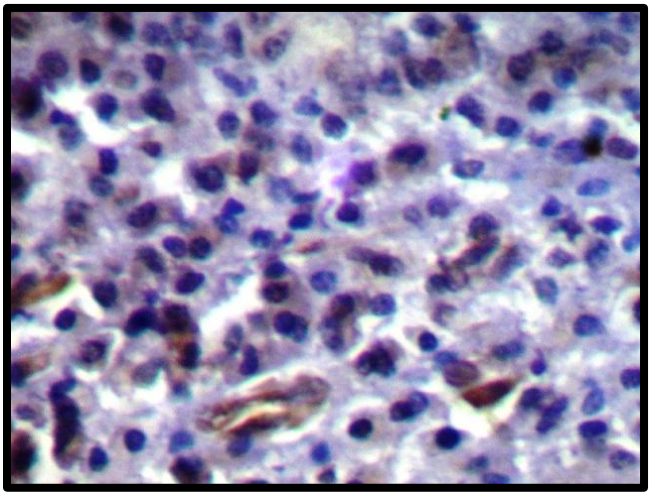


Fig.12: lesions showing low degree of inflammation

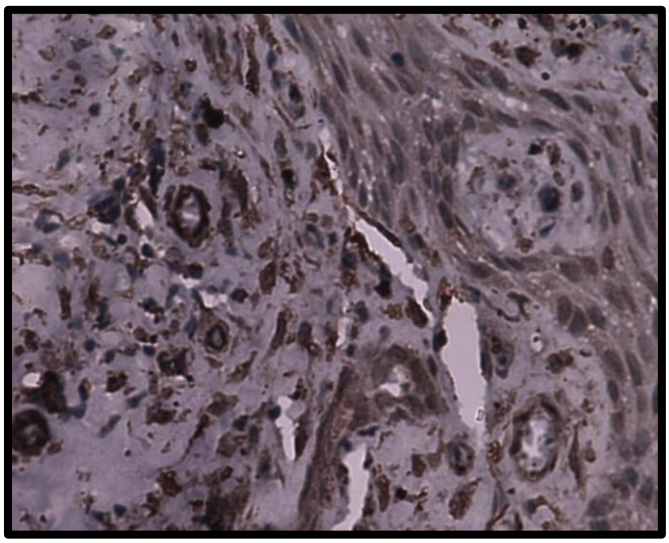


Fig.13: Lesions showing high degree of inflammation

High OSM expression was observed in lesion showing high degree of inflammation. OSM positive cells were stained brown

RESULTS

At light microscopic examination, all specimens revealed the morphological characteristics of apical periodontitis lesions. One section from each specimen was stained with eosin and hematoxylin to evaluate the magnitude of inflammation at the histological level. Each specimen was graded at 40X magnification as, grade low: inflammatory cells less than 50% per field and grade high: inflammatory cells higher than 50% per field. Grading of each specimen was based on the average inflammatory condition in 3 consecutive microscopic fields.

Processed immunohistochemically for OSM expression, sections graded as low were represented by positive stained cells less than 50% per field. Sections graded high exhibited positive stained cells over 50% cells. Grading of each specimen was based on the average OSM stained cells in 3 consecutive microscopic fields. On examining the hematoxylin and eosin stained specimens, various degrees of inflammatory cell infiltration was observed. Among 30 specimens, 15 specimens exhibited high degree of inflammation and 15 specimens exhibited low degree of inflammation.

- Value 1 is given for specimens graded high.
- Value 0 is given for specimens graded low.

All the samples stained positive for OSM. The data obtained from immunohistochemical analysis of group1 were entered in table 1. 11 samples showed a high degree of inflammation and high OSM expression.

The data obtained from immunohistochemical analysis of the group2 were entered in table 2. 4 samples showed a high degree of inflammation and high OSM expression.

The results of the present study were subjected to statistical analysis.Fisher's exact test (two tail) was applied for the statistical analysis of the results. The Fisher's Exact test procedure calculates an exact probability value for the relationship between two dichotomous variables, as found in a two by two cross table. The program calculates the difference between the data observed and the data expected. This analysis is mainly used when the sample size is smaller. A P-value of <0.05 was considered to be statistically significant.

Table 3 and Graph1 showed increased OSM expression in lesions showing high degree of inflammation compared to lesion with low degree of inflammation.

Table 4 and Graph 2 showed increased OSM expression in lesions greater than 5mm compared to smaller lesions.

Table 5 and Graph 3 showed the rank orders of OSM positively stained cells- inflammatory cells (86.7%) > endothelium (73.3%) > epithelium(60%) > Fibroblasts(23.3%).

Results of the present study show that lesions of size greater than 5mm were detected with a statistically significant increase in OSM expression compared to smaller lesions (P-0.011). Also it has been shown that OSM expression was increased with increased degree of inflammation (P-0.001).

Table 1: Lesions of size greater than 5mm

S.NO	CASE NO.	GRADING (degree of inflammation)	GRADING II (OSM expression)	STAINED CELLS			
				FIBROBLASTS	EPITHELIUM	ENDOTHELIUM	INFLAMATORY CELS
1	4430	1	1	+	+	-	+
2	4396	1	1	+	-	+	+
3	4392	1	1	-	+	-	+
4	4440	0	1	-	+	+	-
5	4363	1	1	-	-	+	+
6	4434	1	1	-	+	+	+
7	4400	1	0	-	+	+	+
8	4397	1	1	-	+	+	+
9	4390	1	0	-	+	+	+
10	4417	1	1	-	-	+	+
11	4450	1	1	-	+	-	+
12	4412	0	0	-	+	+	+
13	4356	0	1	-	+	-	+
14	4281	1	1	-	-	+	+
15	4345	0	0	-	-	+	+

Table 2: Lesions of size less than 5 mm

S.NO	CASE NO.	GRADING (degree of inflammation)	GRADING II (OSM expression)	STAINED CELLS			
				FIBROBLASTS	EPITHELIUM	ENDOTHELIUM	INFLAMATORY CELLS
1	4364	0	0	+	+	+	+
2	4365	0	0	-	+	+	+
3	4366	1	1	+	-	-	+
4	4438	1	1	+	+	+	-
5	4409	0	0	-	-	+	+
6	4389	0	0	-	+	-	+
7	4439	0	0	-	-	+	+
8	4410	0	0	+	-	+	+
9	4416	0	1	-	+	+	+
10	4449	0	0	+	-	+	-
11	4322	0	0	-	+	-	+
12	4342	1	1	-	-	+	+
13	4461	0	0	-	+	-	+
14	4376	0	0	-	+	+	+
15	4413	1	0	-	-	+	-

Statistical Analysis

Fisher’s exact test (two tail) was applied for the statistical analysis of the results.The Fisher's Exact test procedure calculates an exact probability value for the relationship between two dichotomous variables, as found in a two by two cross table. The program calculates the difference between the data observed and the data expected. This analysis is mainly used when the sample size is smaller. A P-value of <0.05 mm was considered to be statistically significant

Table 3: OSM expression with degree of inflammation

		GRADING I (Degree of inflammation)				Total		χ2-Value	P-Value
		High		Low		N	%		
		N	%	N	%				
GRADING II (OSM expression)	High	12	80.0	3	20.0	15	50.0	10.800	0.001
	Low	3	20.0	12	80.0	15	50.0		
Total		15	100.0	15	100.0	30	100.0		

Table 4: OSM expression with size of the lesion

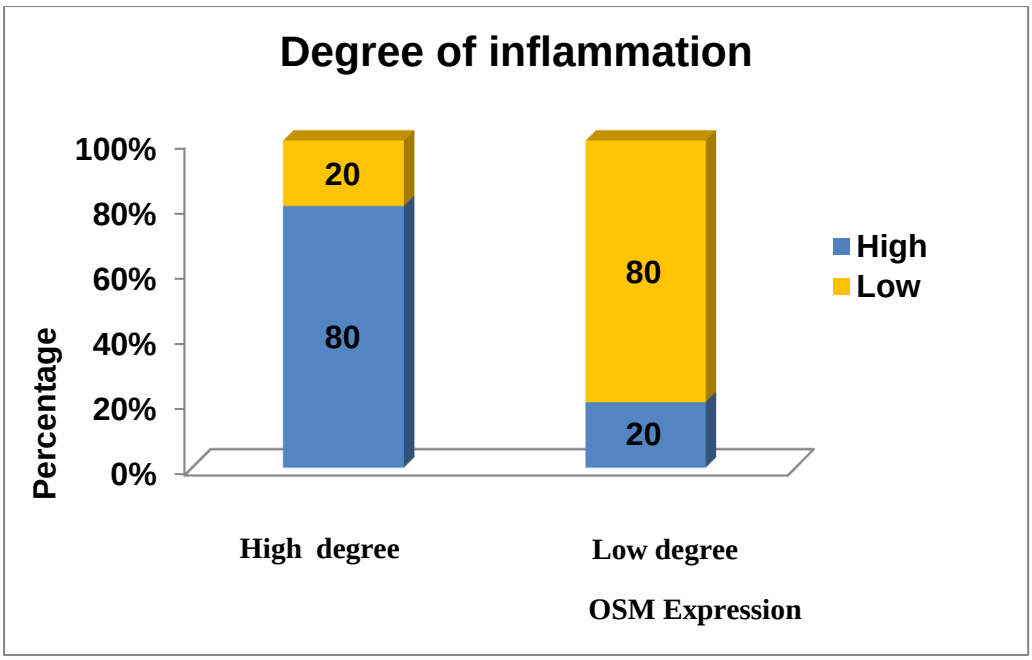
		IMMUNO HISTO CHEMISTRY - Lesion Size				Total		$\chi 2$ - Value	P - Value
		<5 mm		>5 mm					
		N	%	N	%				
GRADING II (OSM expression)	High	4	26.7	11	73.3	15	50.0	6,533	0.011
	Low	11	73.3	4	26.7	15	50.0		
Total		15	100.0	15	100.0	30	100.0		

Table 5: Cells that have stained positive for OSM and their total percentage

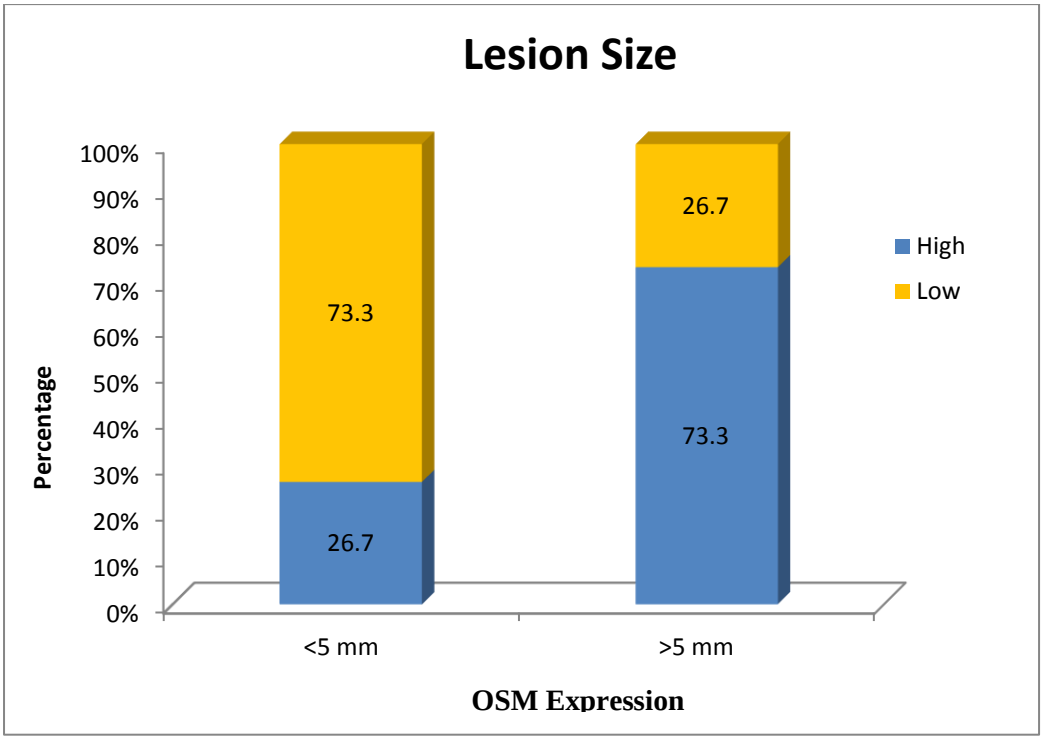
		IMMUNO HISTO CHEMISTRY – Lesion Size				Total		$\chi 2$ – Value	P – Value
		<5 mm		>5 mm		N	%		
		N	%	N	%				
FIBROBLASTS	Positive	5	33.3	2	13.3	7	23.3	1.677	0.390
	Negative	10	66.7	13	86.7	23	76.7		
EPITHELIUM	Positive	8	53.3	10	66.7	18	60.0	0.556	0.456
	Negative	7	46.7	5	33.3	12	40.0		
ENDOTHELIUM	Positive	11	73.3	11	73.3	22	73.3	0.000	1.000
	Negative	4	26.7	4	26.7	8	26.7		
INFLAMMATORY CELLS	Positive	12	80.0	14	93.3	26	86.7	1.154	0.598
	Negative	3	20.0	1	6.7	4	13.3		
Total		15	100.0	15	100.0	30	100.0		

To summarize the results:-

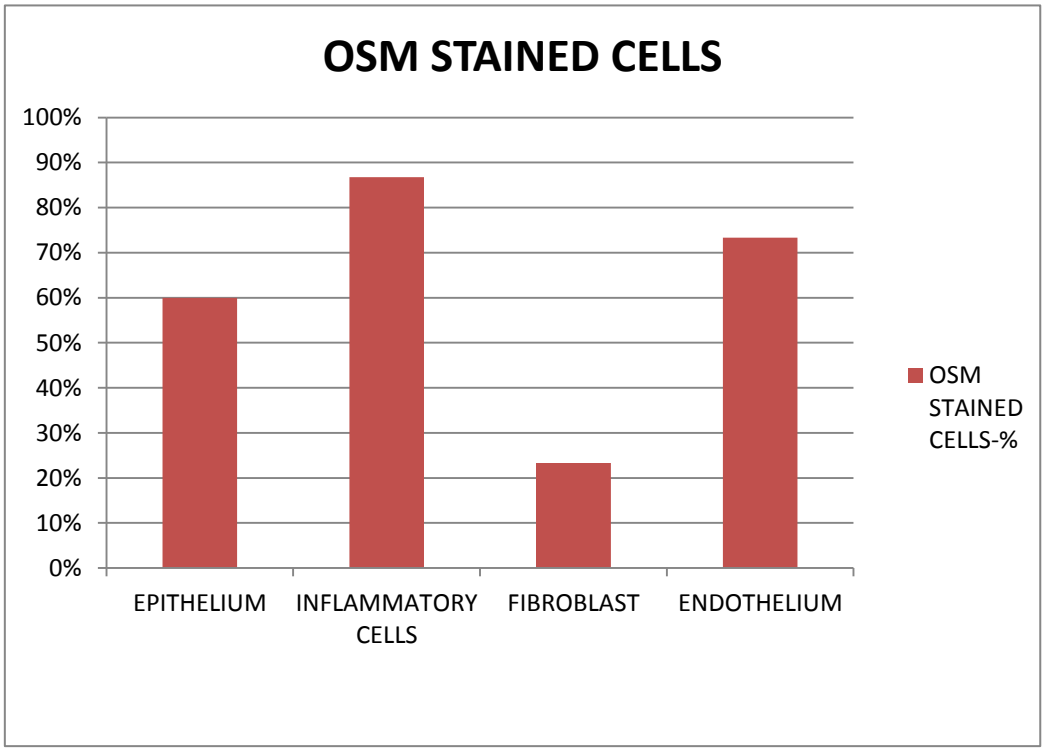
1. Increased OSM expression seen in lesions showing high degree of inflammation compared to lesion with low degree of inflammation.
2. Increased OSM expression seen in lesions greater than 5mm compared to smaller lesions.
3. The rank orders of OSM positively stained cells- inflammatory cells (86.7%) > endothelium (73.3%) > epithelium (60%) > Fibroblasts (23.3%).



Graph 1: Comparison of OSM expression with degree of inflammation



Graph 2: Comparison of OSM expression with size of the lesion



Graph 3: Percentage of OSM stained cells

DISCUSSION

Apical periodontitis is an inflammatory disorder of periradicular tissues caused by persistent microbial infection in the root canal system of the affected tooth (Kakehashi et al. 1965, Sundqvist 1976). Initially the tooth pulp becomes inflamed and necrotic by an autogenous oral microflora. The infected and necrotic pulp offers a selective habitat for the organisms (Fabricius et al. 1982).

The endodontic environment provides a selective habitat for the establishment of a mixed, predominantly anaerobic flora. The presence of several distinct types of bacteria in the necrotic dental pulp was demonstrated more than a century ago (Miller, 1890). The essential role of micro-organisms in the etiology of apical periodontitis nevertheless remained uncertain for many years. Half a century later, it was shown (Kakehashi et al., 1965) that no apical periodontitis developed in germ-free rats when their molar-pulps were kept exposed to the oral cavity, as compared with control rats with a conventional oral microflora in which massive periapical radiolucencies occurred.[15]

The great significance of obligate anaerobes in endodontic infections was soon established (Möller, 1966). A series of pathobiological studies (Fabricius, 1982) subsequently determined: (a)

the conditions under which the endodontic flora develops and establishes itself, and (b) the biological properties and endodontic conditions which may favor the root canal flora to become pathogenic.

The root canal flora of infected, untreated teeth is dominated by obligate anaerobes, usually belonging to the genera fusobacterium, porphyromonas, prevotella, eubacterium and peptostreptococcus. Culture studies (Waltimo et al., 2003) and the application of scanning electron microscopy (Sen et al., 1995) have revealed the presence of fungi in canals of teeth with primary apical periodontitis. The presence of intraradicular viruses has so far been shown only in non-inflamed dental pulps of patients infected with immuno-deficiency virus.[23] Culture studies show that the root canal flora of previously root filled teeth contained a very few species of bacteria, which is predominated by E.faecalis.[39]

The microbes grow in sessile biofilms, aggregates, coaggregates, and also as planktonic cells suspended in the fluid phase of the canal (Nair 1987). A biofilm (Costerton et al. 2003) is a community of microorganisms embedded in an exopolysaccharide matrix that adheres onto a moist surface whereas planktonic organisms are free-floating single microbial cells in an aqueous environment. Bacterial aggregations

in the biofilm have been thought to be the cause of therapy-resistant apical periodontitis. Microorganisms protected in biofilms are greater than one thousand times more resistant to biocides as the same organisms in planktonic form (Wilson 1996, Costerton & Stewart 2000).[39]

Essentially, this habitat-adapted polymicrobial community residing in the root canal has several biological and pathogenic properties, such as antigenicity, mitogenic activity, chemotaxis, enzymatic histolysis, and activation of host cells. The microbes in the root canal can advance, or their products can egress, into the periapex. In response, the host mounts an array of defenses consisting of several classes of cells, intercellular messengers, antibodies, and effector molecules. The microbial factors and host defense forces encounter, clash with, and destroy much of the periapical tissue, resulting in the formation of various categories of apical periodontitis lesions.

In spite of the formidable defense, the body is unable to destroy the microbes most of which are well entrenched in the sanctuary of the necrotic root canal, which is beyond the reaches of body defenses. Therefore, apical periodontitis is not self-healing.[23] The treatment of apical periodontitis consists of eliminating infection from the root canal

and preventing re-infection by a hydraulic seal of the root canal space. Nevertheless, endodontic treatment can fail for various reasons.

The goal of the root canal treatment consists of eradicating the root canal microbes or substantially reducing the microbial load and preventing re-infection by root canal filling (Nair et al. 2005). When the treatment is done properly, healing of the periapical lesion usually occurs with hard tissue regeneration that is characterized by reduction of the radiolucency on follow-up radiographs. Nevertheless, a complete healing of calcified tissues or reduction of the apical radiolucency does not occur in all root canal treated teeth.[22] The presence or absence of signs and symptoms (pain, swelling and sinus tract) and the PAI scores were used as outcome measures.

Periapical index (PAI) scores provides an ordinal scale of five scores ranging from "healthy" to "severe periodontitis with exacerbating features". The PAI is based on reference radiographs with verified histological diagnosis.[14] The PAI scores:

1. Normal periapical structures
2. Small changes in bone structure
3. Changes in bone structure with some mineral loss
4. Periodontitis with well defined radiolucent area
5. Severe periodontitis with exacerbating features

Treatment was considered a success when clinically, there was absence of signs and symptoms and radiographically the PAI score was 1 or 2 or there was a typical scar tissue formation. Treatment was considered a failure when clinical signs and symptoms were present or when the PAI score was 3 or higher.[1] Periapical radiolucencies persist when treatment procedures have not reached a satisfactory standard for the control and elimination of infection.

The prolonged healing process of many periapical lesions has been attributed to the activated macrophages in the lesion that may maintain their state of activation long after the initial cause of their activation has been eliminated by root canal treatment; namely, the activation state may outlive its useful purpose and become a burden by inhibiting resolution of the lesion. The production of bone-resorbing cytokines by these cells may persist for many months after the completion of the root canal treatment, thus preventing resolution of the periapical bone defects.[19] OSM production by activated macrophages and the bone resorbing property of this cytokine could be one of the possible reason for persistent periapical periodontitis lesions.

Oncostatin M (OSM) is a multifunctional cytokine belonging to the Interleukin (IL)-6 family [1] which also includes IL-11, IL-27,

IL-31, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC) and neuropoietin (NP).[4]OSM regulates cell growth and differentiation in a wide variety of biological systems, including hematopoiesis, neurogenesis, and bone homeostasis.[5] Hence OSM is regarded as a pleiotropic cytokine since it participates in a variety of physiological processes. Marc Vasse analysed the effect of OSM on angiogenesis and found that OSM can induce an angiogenic effect on the capillary endothelial cellswhich could be implicated in the pathological process of atherosclerosis.[35] Gomez suggested the potential role of OSM in the regulation of complex cellular processes such as gene activation, cell survival, proliferation and differentiation.[7]Human OSM is a secreted glycoprotein which is initially translated as a 252 amino acid polypeptide with a 25 residue hydrophobic signal sequence at the N-terminus that is removed during the secretion process.[6]

OSM was first identified in the human myelocytic leukemia cell line U937, after treatment with phorbol esters. Subsequent studies identified activated T lymphocytes and macrophages, cell cultures derived from acquired immunodeficiency virus (AIDS)-related Kaposi's sarcoma (Nair et al., 1992) and retrovirus-infected CD4 T-cells as sources of OSM mRNA. OSM secretion occurs through a 2 step

mechanism in Polymorphonuclear neutrophils, consisting of early release of a preformed stock followed by de novo protein synthesis.[9]

Glycoprotein 130(gp130) is a receptor subunit capable of intracellular signaling that are required for the cellular action of a wide range of cytokines.[28] Every cytokine that binds to gp130 generates specific intracellular signaling events by forming specific receptor:ligand complexes. IL-6 cytokine family members, sharing this common signal transducer gp130, has been found to be responsible for bone remodeling.[11]Activation of gp130 by these cytokines occurs through different mechanisms. IL-6, IL-11, and IL-27 activate gp130 after binding to specific nonsignaling receptor subunits, which then trigger the homodimerization and activation of gp130.[2]

In contrast, cytokines including LIF and OSM engage gp130 to form a heterodimer interaction with one of two gp130-related proteins, LIFR or OSMR. These distinct modes of gp130 activation may provide a molecular basis for the pattern of cellular events orchestrated by these related factors .[10] OSM signals through 2 different receptors in humans. [1] LIF/OSM shared receptor which shares high-affinity binding with LIF, and [2] the OSM-specific receptor which binds OSM uniquely.[21] Leukemia inhibitory factor(LIF), an evolutionarily related protein is

structurally similar to OSM and both have similar biologic activities on a variety of target cells, including hematopoietic cells, hepatocytes, neurons and bone cells.[3]Based on the action of LIF & OSM in bone cultures, these cytokines are regarded as the potent remodeling agents with pleiotropic effects on bone formation and resorption.[34]

OSM has been found to enhance the differentiation and proliferation of osteoblasts during bone development and also induces the formation of osteoclasts that contribute in bone homeostasis.[18] OSM stimulate collagenase-3 and tissue inhibitors of metalloproteinases expression in osteoblasts and these effects may be involved in mediating the bone remodeling action of this cytokine.[34] In accordance with studies performed by Cawston et al, Hui et al it has been suggested that OSM appears to be an important cofactor with other pro-inflammatory cytokines such as IL-1, TNF-α and IL-17 in mediating cartilage/bone destruction. When these pro-inflammatory cytokines are over expressed in combination with OSM in murine joints, a marked increase in damage to the joint tissues is observed. In contrast to the information presented here, A.F Wahl presented a study where he showed that OSM can modulate the expression of human acute phase proteins and protease inhibitors and thereby limiting tissue damage at the sites of

inflammation.[36] This may be due to the differences in the sources and purity of the proteins, in addition to the experimental design variations.

RANKL is a TNF superfamily member and an essential mediator of osteoclastogenesis. It is produced from osteoblastic- stromal cells, synovial fibroblasts, chondrocytes and activated T lymphocytes. This TNF-related cytokine and its receptor, RANK, are considered key factors in osteoclast differentiation, and RANK signalling is vital for osteoclast activation and survival. Furthermore, there was increased expression of RANK and its ligand RANKL in the inflammatory cells, in inflamed synovium and in articular cartilage of knee joints treated with the cytokine combinations. In vivo, oncostatin M in combination with either IL-1 or tumor necrosis factor alpha represents cytokine combinations that promote bone destruction.[13]

The expression of increased OSM has been demonstrated in inflammatory bone diseases like rheumatoid arthritis and osteoarthritis.[12] Increased expression of OSM has also been detected in chronic periodontitis. S.J.Lin et al established a possible modulatory role for OSM in the inflammatory cascade of chronic periodontitis.[17] Manoj Kumar et al measured the levels of OSM in the gingival crevicular fluid and serum of the chronic periodontitis patients. He found a significant

correlation between the levels of OSM in serum and gingival crevicular fluid. [31] Increased OSM level both in gingival crevicular fluid and serum, and the decreased levels after initial periodontal therapy may suggest use of OSM as an inflammatory biomarker in the periodontal disease.

Clinically it has been demonstrated that OSM expression has been detected in advanced apical periodontitis lesions. Tsai et al demonstrated expression of OSM in epithelialised apical periodontitis lesions. He concluded that OSM forms a part of the cytokine network that is involved in the disease process of apical periodontitis lesions. OSM can be regarded as the cytokine responsible for the expansion of these lesions inside the bone.[33]

However the presence of OSM in smaller apical periodontitis lesions with minor alterations in the bone structure (i.e) with non-extensive bone destruction has not been studied. Hence the aim of the present study is to investigate the expression of OSM in apical periodontitis lesions. The objective of the present study is to investigate the expression of OSM in apical periodontitis lesions and to compare the expression of OSM in lesions of size greater than 5mm and lesions of size less than 5mm.

In our study 30 periapical lesions of pulpal origin were collected with informed consent from the patient at the time of apical surgery. Exclusion criteria included the patients with inflammatory bone diseases like rheumatoid arthritis, osteoarthritis and patients with chronic periodontitis[37]. These cases are excluded because the studies conducted by Hui et al (1997) had shown increased levels of oncostatin-M expression in inflammatory diseases like rheumatoid arthritis, osteoarthritis and chronic periodontitis. All the patients were without systemic diseases and with radiographic evidence of periapical lesions including periradicular alveolar bone loss. Initially non surgical endodontic treatment was attempted in these patients but the treatment has failed.

Most of the patients were symptomatic with persistent pain and with radiographic evidence of non -healing periapical lesions. Age of the patient is an important inclusion criteria in this study. Age group was standardized in the range between 20-40yrs because in this period patients are less susceptible to systemic diseases. Paralleling cone radiographic technique was followed to standardize radiographic procedures.

The collected periapical lesions were divided into 2 Groups, Group I (n=15) samples which contained lesions of size >5mm and Group II (n=15) samples which contained lesions of size <5mm. The grouping was done based on the size of the lesion. The classical Toronto study performed by Wang et al (2004) revealed that lesion size is a major predictor of outcome of apical surgery. Better treatment outcome was observed in teeth with smaller lesions (<5mm) compared to larger lesions. [38] Since size of the lesion is a major predictor of success, in the present study grouping was done based on this factor. Group I consisted of lesions of size greater than 5mm and group II consisted of lesions of size less than 5mm. The collected samples were then preserved. Because of the superior preservation of morphology, formalin- fixed paraffin-embedded (FFPE) tissue remains today the medium of choice for most clinical and research studies.[41] The collected tissue specimens were fixed with 10% buffered formalin overnight and dehydrated in an ascending series of graded alcohol. Finally the fixed, dehydrated specimens were embedded in paraffin and made in to blocks.

However, the loss of immunoreactivity by many antigens caused as a result of fixation in formalin has introduced many challenges.An entirely new approach for the restoration of immunoreactivity in FFPE tissue sections was reported by Shi et al 1991. However, another major

step forward in the use of heat was reported by Cattoretti et al. Based on his technique, in the present study after deparaffinizing and rehydrating the tissue sections, the slides are immersed in an aqueous solution commonly referred to as a "retrieval solution".[27] The solution used here is citrate buffer at a pH of 6. The slides were then transferred to citrate buffer and autoclaved for 30 minutes at 15lbs pressure.

In this study indirect staining technique was used where an unconjugated primary antibody first binds to the antigen. The primary antibody (Rabbit antihuman SC-129, santacruz Biotechnology) used in this study was diluted with phosphate buffered saline at a ratio of 1:50. An enzyme-labelled secondary antibody directed against the primary antibody (now the antigen) is then applied, followed by the substrate-chromogen solution. In this study, Diaminobenzidine is used as the chromogen substrate for localizing the antibody binding.[32]

If the primary antibody is made in rabbit or mouse, the secondary antibody must be directed against rabbit or mouse immunoglobulins, respectively. This method is versatile because a variety of primary antibodies from the same species can be used with the same conjugated secondary antibody. The procedure is also more sensitive as several secondary antibodies are likely to react with a number of different

epitopes of the primary antibody thus amplifying the signal as more enzyme molecules are attached per each target site.[41]

Another section is made from each specimen and stained with hematoxylin and eosin (H & E). The sections were stained with hematoxylin and eosin to evaluate the magnitude of inflammation at the histological level.

In the present study all the samples stained with anti-OSM antibody showing the generalized presence of the OSM cytokine in all the cases with persistent periapical periodontitis. Both OSM stained slides and eosin-hematoxylin stained slides were observed under light microscopy at 40 X and graded. Grading of the samples were performed similar to the study done by Tsai et al (2008). H & E stained slides were graded to evaluate the degree of inflammation. Grade low- inflammatory cells less than 50% per field. Grade high-inflammatory cells greater than 50% per field. Grading of each specimen was based on the average inflammatory condition in 3 consecutive fields.

Immunohistochemically stained slides were graded for the expression of OSM which will take up a brown stain. Immunohistochemically processed slides were graded for OSM expression in a similar manner as described above. Sections graded low

were represented by positive stained slides less than 50% of the total cells. Sections were graded high when the positive stained cells were greater than 50% per field. Grading of each specimen was based on the average positive cell expression in 3 consecutive fields.

Since the sample size was smaller, Fisher's exact test was applied for statistical analysis of the results. A P-value < 0.05% was considered to be statistically significant. Statistical analysis of the results showed a significantly higher OSM expression in group I [lesions of size greater than 5 mm] compared to group II[lesions of size less than 5mm] P=0.011. Differences in OSM expression between tissues with high and low levels of inflammation were subsequently analysed using Fisher's exact test. A significantly higher OSM expression was noted in lesions with high levels of inflammation compared to tissues with low levels of inflammation. The results indicate a higher OSM expression in larger lesions and lesions showing higher degree of inflammation.

OSM expression was found in epithelium, inflammatory infiltrates, endothelium and connective tissue. The rank orders with respect to OSM positively stained cells were found as follows: inflammatory infiltrates (86.7%) > endothelium (73.3%) > epithelium (60%) > fibroblasts (23.3%). OSM is not expressed in negative controls

of apical periodontitis lesions. In inflamed gingival specimens OSM was detected in epithelium, endothelium, fibroblasts and inflammatory infiltrates. The expression of OSM might play a role in the pathogenesis of apical periodontitis lesions.[33]

The mechanism responsible for OSM expression in apical periodontitis lesions can be explained as follows. Apical periodontitis lesions are believed to result from continuous antigenic stimulation from inflamed or necrotic root canals. A classical study performed by kakehashi et al in 1965 showed an association between bacteria and endodontic infection. According to a study performed by Suda et al (2002) OSM was also found to be synthesized by bacterial lipopolysaccharides in dendritic cells. He suggested that OSM produced by dendritic cells at infectious sites may play a role in modulating inflammatory responses.[30] Therefore OSM expression in apical periodontitis lesions may be induced directly by the bacteria from necrotic and infected pulps or indirectly by the inflammatory mediators generated by resident cells. Thus these cells may play an important role in the pathogenesis of apical periodontitis lesions by controlling the synthesis of inflammatory mediators and OSM.

Palmqvist et al (2002) implicated that OSM is a mediator of bone destruction.[24] OSM was reported to induce tissue type Plasminogen Activator (t-PA) expression in vitro.[29] Plasminogen activating system participates in degradation of bone matrix. Stimulation of osteoclast differentiation in vitro by OSM has also been studied (Richards et al).[25] Apical periodontitis lesions are believed to result from continuous antigenic stimulation from inflamed and necrotic root canals.

As the periapical lesions tend to grow within the jaw bone, it is understandable that expansion of the lesion is possible only with bone destruction. Bone expansion occurs by activated osteoclasts at the lesion site. As OSM participate in bone degradation, taken together it is hypothesized that the possible mechanism by which lesion growth occurs within the jaw bone is through OSM expression pathway. The results of the present study correlates with the above mentioned explanation that increased OSM expression is seen in larger lesion compared to smaller lesion. Increased OSM presence was seen in growing lesions.

OSM has received wide attention because of its participation in diversified physiological processes. Tsai et al demonstrated the role of OSM in the expansion of epithelialized apical periodontitis lesions. The

findings of our study indicated that OSM is not only responsible for the growth of the lesions but also it could be one of the possible reasons which actually delays the healing process. Goren proved that increased OSM expression is functionally connected to the polymorphonuclear neutrophil infiltration and associated with impaired healing in chronically inflamed lesions.[8] All the cases selected in our study were refractory to nonsurgical endodontic procedure. In our study there is generalized presence of OSM irrespective of the size of the lesion. This strongly suggests OSM expression by the resident cells is mainly responsible for the impaired bone deposition at the lesion site and as a consequence delayed healing. By performing periapical surgery and elimination of the residing inflammatory periapical tissue will help in elimination of OSM and thereby accentuates healing mechanisms.

In addition increased OSM expression is seen in lesions greater than 5mm compared to smaller lesions, which could be the possible cause for extensive bone destruction in larger lesions. This could also be the reason for better treatment outcome observed in smaller (≤5mm) lesions compared to larger lesions after apical surgery.[38] The expression of OSM increases with the grade of inflammation in apical periodontitis lesions showing that the resident cells [inflammatory infiltrates,

endothelium, epithelium and fibroblasts], act as a reservoir of OSM that could be secreted at certain stages of inflammation.

In the present study OSM was detected in all the periapical lesions that are persistent after initial endodontic therapy. Presence of OSM could be the possible reason behind the failure of non surgical endodontic management. Increased OSM expression was observed in lesions of size greater than 5mm and in lesions showing high degree of inflammation. Thus the role of OSM in the pathogenesis of apical periodontitis lesion is worthy of further investigation.

SUMMARY

The purpose of the study is to investigate the expression of Oncostatin M (OSM) in apical periodontitis lesions and to compare the expression of OSM in lesions of size greater than 5mm and lesions of size less than 5mm. OSM is a pleiotropic cytokine which has a role in the pathogenesis of many inflammatory diseases and brings about bone resorption.

In our study, 30 periapical lesions of pulpal origin were collected with informed consent from the patient at the time of apical surgery. Exclusion criteria included the patients with inflammatory bone diseases like rheumatoid arthritis, osteoarthritis and patients with chronic periodontitis. All the patients are without systemic diseases and with radiographic evidence of periapical lesions including periapical alveolar bone loss. Initially non surgical endodontic treatment was attempted in these patients but the treatment has failed. Age of the patient is an important inclusion criteria in this study. Age group was standardized in the range between 20-40yrs, because in this period patients are less susceptible to systemic diseases. Paralleling cone radiographic technique was followed to get the accurate mesio-distal dimension of the lesion.

The collected periapical lesions were divided into 2 Groups, Group I (n=15) samples and Group II (n=15) samples. The grouping was done based on the size of the lesion. Group I consisted of lesions of size greater than 5mm and group II consisted of lesions of size less than 5mm. the collected samples were then preserved. Because of the superior preservation of morphology, formalin- fixed paraffin-embedded (FFPE) tissue remains today the medium of choice for most clinical and research studies. The collected tissue specimens were fixed with 10% buffered formalin overnight and dehydrated in an ascending series of graded alcohol. Finally the fixed, dehydrated specimens were embedded in paraffin and made in to blocks.

5 micron thin sections were made from the paraffin blocks and the sections were transferred to the slides. The slides were processed immunohistochemically to locate the OSM antigen. Another 5 micron section from the samples were made and stained with eosin and hematoxylin to assess the magnitude of inflammation. The slides were then observed under light microscope at 40 X and graded.

Results of the present showed that all the samples stained positive for OSM. Increased OSM expression was observed in group1 (i.e) lesions greater than 5 mm compared to smaller lesions. In addition increased

OSM expression was observed in lesions showing high degree of inflammation. The results of the present study agree with the study performed by Tsai et al (2008) where OSM expression was increased with increase in the degree of inflammation.

The OSM has received considerable attention because of its participation in many inflammatory diseases. OSM could be detected in areas of the chronic apical periodontitis lesions, suggesting that OSM, stored in the cytosol of epithelial cells, inflammatory, cell, fibroblasts, and endothelial cells, may represent a reservoir of OSM activity that can be released at certain stages of the inflammatory reaction. In addition, the expression of OSM increases with the grade of inflammation in apical periodontitis lesions. Thus the role of OSM in the pathogenesis of apical periodontitis lesion is worthy of further investigation.

CONCLUSION

Within the limitations of the present study, it can be concluded that

(i) All the tissue specimens obtained from periapical surgery stained with anti OSM antibody showing the generalized presence of the OSM cytokine in cases with persistent periapical periodontitis.

(ii) OSM forms the cytokine network that is responsible for the bone resorption associated with periapical lesions.

(iii) Immunoreactivity of OSM was expressed in epithelium, endothelium, inflammatory infiltrates and fibroblasts. The OSM signal was expressed in the following rank order- inflammatory infiltrates (86.7%) > endothelium (73.3%) > epithelium (60%) > fibroblasts (23.3%)

(iv) Increased OSM expression was observed in lesions greater than 5mm compared to smaller lesions.

(v) Increased OSM expression was observed in lesions with increased degree of inflammation.

(vi) Increase in OSM expression can denote the increase in degree of inflammation of periapical lesions.

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